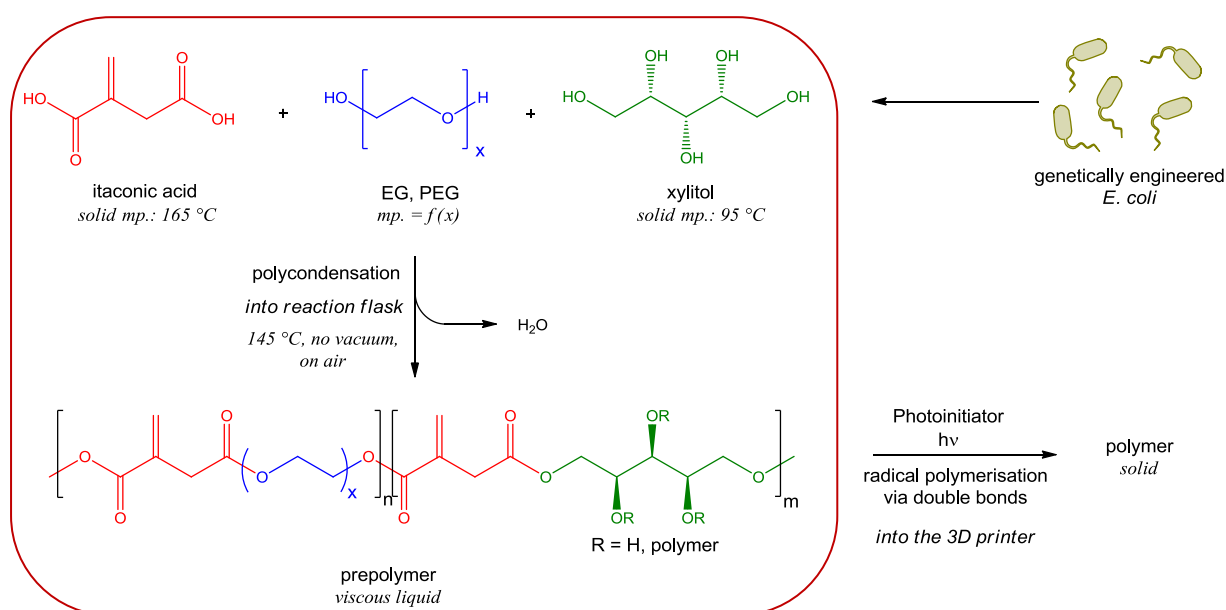


iGEM Chemistry Group - Protocol



Members of the Chemistry Group:

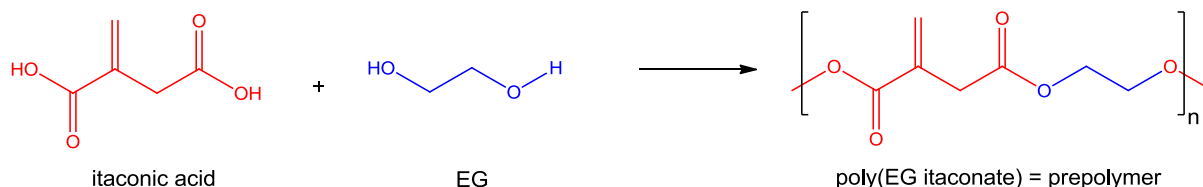
Anastasia Weyrich (AW), Fabian Rohden (FR), Stefan Zens (SZ), Sebastian Jäger (SJ),

Table of Contents

Synthesis of prepolymers and characterization	3
Thermal polyesterification of itaconic acid (IA) and ethylene glycol (EG)	3
Batch A0.....	3
Batch A14.....	4
Thermal polyesterification of itaconic acid (IA), polyethylene glycol 400 (PEG-400)	5
Batch A1.....	5
Batch A2.....	6
Batch A3.....	7
Batch A4.....	9
Batch A10.....	11
Thermal polyesterification of itaconic acid , polyethylene glycol 400 and xylitol	12
Batch A5.....	13
Batch A6.....	14
Batch A8.....	15
Batch A9.....	16
Thermal polyesterification of itaconic acid (IA) and polyethylene glycol 200 (PEG-200).....	17
Batch A7.....	18
Batch A11.....	19
Batch A12.....	20
Batch A13.....	21
Batch A15.....	22
Analysis of the prepolymers with GPC	24
Curing of prepolymers.....	25
References:	27
Appendix	28
Pictures of prepolymers during and after synthesis.....	28
Pictures of cured prepolymers	30

Synthesis of prepolymers and characterization

Thermal polyesterification of itaconic acid (IA) and ethylene glycol (EG)



Polymers were prepared in dependence on the experimental procedures of *Barrett et al.*^[1]

The procedure requires exact masses. Therefore an analytical balance (4 decimal places) was used and if not otherwise stated EG as well as itaconic acid were pre-dried before use on a high vacuum line ($< 10^{-3}$ mbar) overnight.

To avoid contamination with oxygen during the reaction and to remove produced water, common Schlenk technique was applied with Argon as inert gas (A0-A2). Batch A3 was performed under atmospheric conditions instead of Schlenk technique and showed good results. From that point onwards all experiments were performed under atmospheric conditions. This suited our application scenario well (see application scenario for further details).

Batch A0

Date: 31/05/15 – 01/06/15

Lab members: AW, SZ

Preparation process:

A 20 mL snap-cap vial was loaded with 1.6155 g (26.027 mmol, 1 eq.) EG and 3.3853 g (26.021 mmol, 1 eq.) itaconic acid and flushed with argon. Within 20 minutes the mixture was heated up to 100 °C with an oil bath and stirred under argon atmosphere. As a colorless melt had formed the vacuum was applied ($3 \cdot 10^{-2}$ mbar) and the mixture was stirred at 100 °C for 19 h.

Visual Observations:

The yellow-colored polymer is a rigid gel and not applicable for 3D printing.

Analysis:

No further analyses were performed.

Batch A14

Date: 12/08/15 – 13/08/15

Lab members: SJ

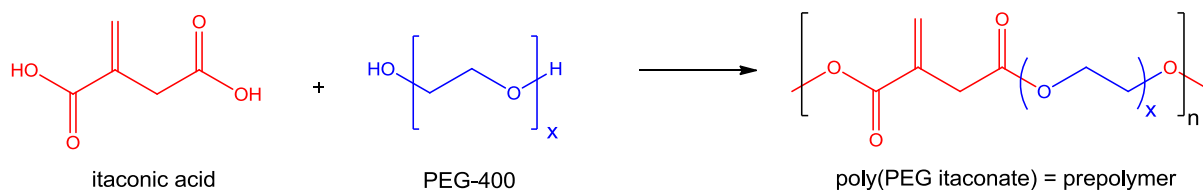
Preparation process:

A 20 mL snap-cap vial was loaded with 1.8156 g (29.283 mmol, 1 eq.) EG and 3.8066 g (29.279 mmol, 1 eq.) itaconic acid. For this experiment itaconic acid was not pre-dried under high vacuum. The vial was capped with a pierced aluminium foil and the mixture was heated up to 145 °C with an oil bath under atmospheric conditions. No vacuum was applied and the mixture was stirred under atmospheric conditions at 145 °C over night.

Visual Observations:

During the reaction a film of ethylene glycol condensed on the vial wall. This is due to the boiling point of EG (197 °C). In this way the exact IA/EG ratio could have changed. The yellow-colored polymer is still rigid gel and not applicable for 3D printing. For a picture of A14 see appendix.

Thermal polyesterification of itaconic acid (IA), polyethylene glycol 400 (PEG-400)



Polymers were prepared in dependence on the experimental procedures of *Barrett et al.*^[1]

The procedure requires exact masses. Therefore an analytical balance (4 decimal places) was used and if not otherwise stated PEG as well as itaconic acid were pre-dried before use on a high vacuum line ($< 10^{-3}$ mbar) overnight.

To avoid contamination with oxygen during the reaction and to remove produced water, common Schlenk technique was applied with Argon as inert gas (A0-A2). Batch A3 was performed under atmospheric conditions instead of Schlenk technique and showed good results. From that point onwards all experiments were performed under atmospheric conditions. This suited our application scenario well (see application scenario for further details).

Batch A1

Date: 02/06/15 – 03/06/15

Lab members: AW, SZ

Preparation process:

A 50 mL Schlenk flask was loaded with 1.213 g (3.033 mmol, 1 eq.) PEG-400 and 0.395 g (3.032 mmol, 1 eq.) itaconic acid and flushed with argon. Within 20 minutes the mixture was heated up to 100 °C with an oil bath and stirred under argon atmosphere. As a colorless melt had formed the vacuum was applied ($3 \cdot 10^{-2}$ mbar) and the mixture was stirred at 100 °C for 17 h. The slightly yellow viscous liquid was cooled down to room temperature.

For a picture of A1 see appendix.

Analysis:

No further analyses were performed.

Batch A2

Date: 09/06/15 – 10/06/15

Lab members: FR, SJ

Preparation process:

A 25 mL Schlenk tube was loaded with 2.169 g (5.422 mmol, 1 eq.) PEG-400, 0.705 g (5.420 mmol, 1 eq.) itaconic acid and capped with a septum. Within an hour the mixture was heated up to 145 °C with an oil bath and stirred under argon atmosphere. As a colorless melt had formed the vacuum was applied ($< 10^{-3}$ mbar) and the mixture was stirred at 145 °C for 24 h. The slightly yellow viscous liquid was cooled down to room temperature.

For a picture of A2 see appendix.

Visual Observations:

During the reaction itaconic acid recrystallised on the flask surface which was not dipped into the oil bath. Therefore the ratio between itaconic acid and PEG-400 had changed.

Analysis:

The product was analyzed by differential scanning calorimetry (DSC) to identify the glass transition (T_g) temperature.

The T_g of A2 is -4.7 °C.

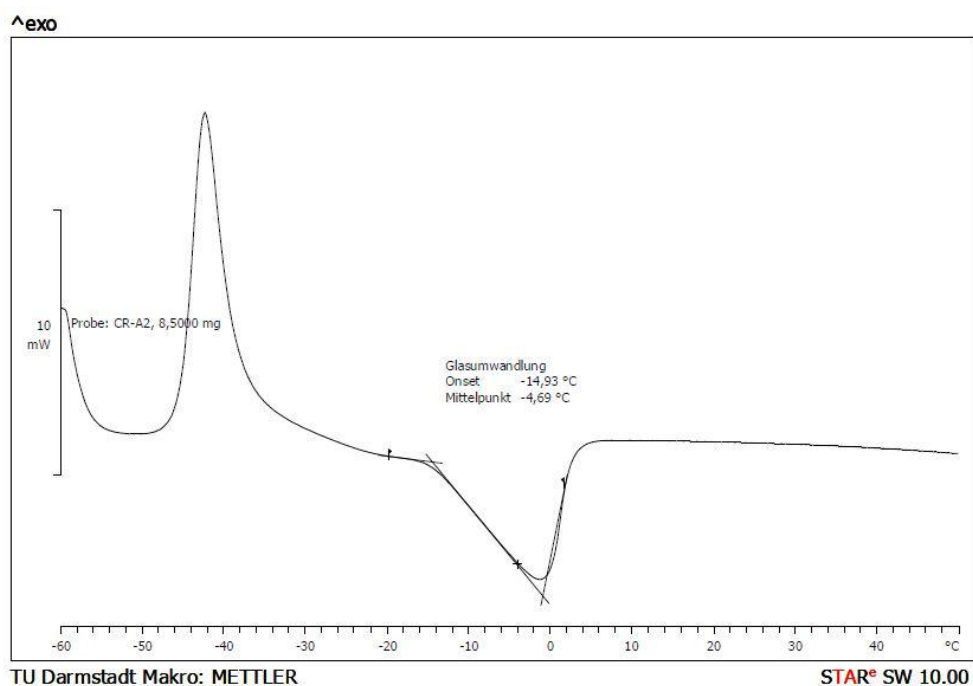


Figure 1: DSC- Plot of A2. The T_g of A2 is -4.7 °C

Batch A3

Date: 11/06/15 – 12/06/15

Lab members: FR, SJ, AW, SZ

Preparation process:

A 10 mL Schlenk tube was loaded with 2.675 g (6.688 mmol, 1 eq.) PEG-400, 0.869 g (6.679 mmol, 1 eq.) itaconic acid and capped with a septum. The mixture was heated up to 145 °C with an oil bath under atmospheric conditions. No vacuum was applied and the mixture was stirred under atmospheric conditions at 145 °C for 24 h. The orange-colored viscous liquid was cooled down to room temperature.

Visual Observations:

The orange-colored product was more viscous than A1 and A2. For a picture of A3 see appendix.

Analysis:

The product was analyzed by differential scanning calorimetry (DSC) to identify the glass transition (T_g) temperature. The T_g of A3 is -45.8 °C.

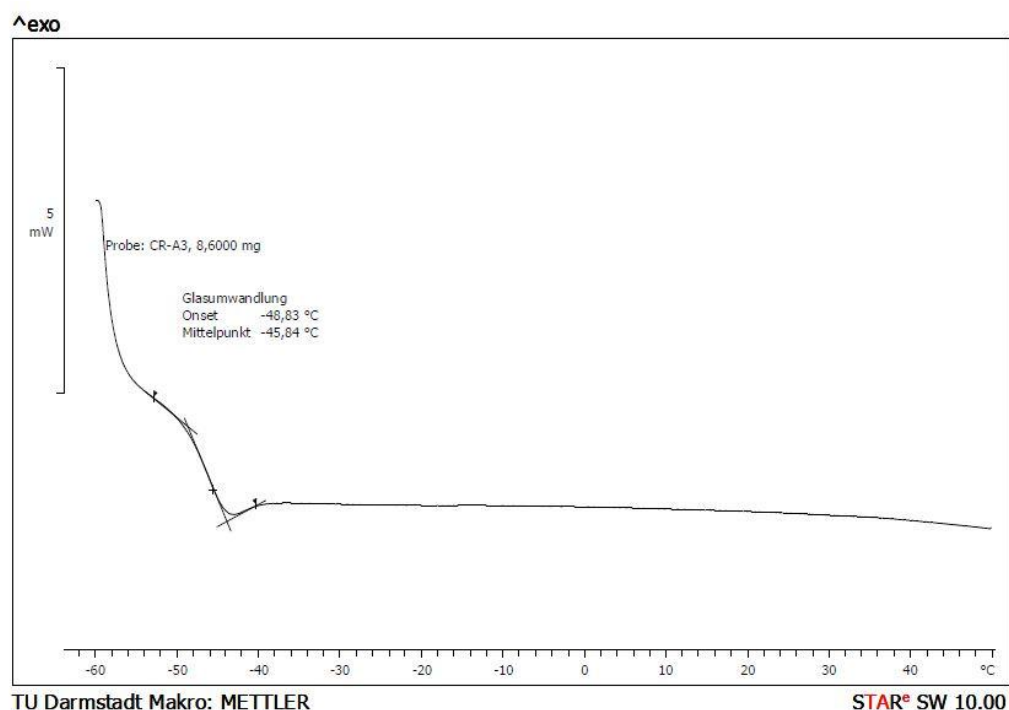


Figure 2: DSC- Plot of A3. The T_g of A3 is -45.8 °C

¹H-NMR

The product was analyzed by ¹H-NMR to ensure that double bonds are retained in the product.

Figure 3 shows the spectrum with an enlargement of the olefin region.

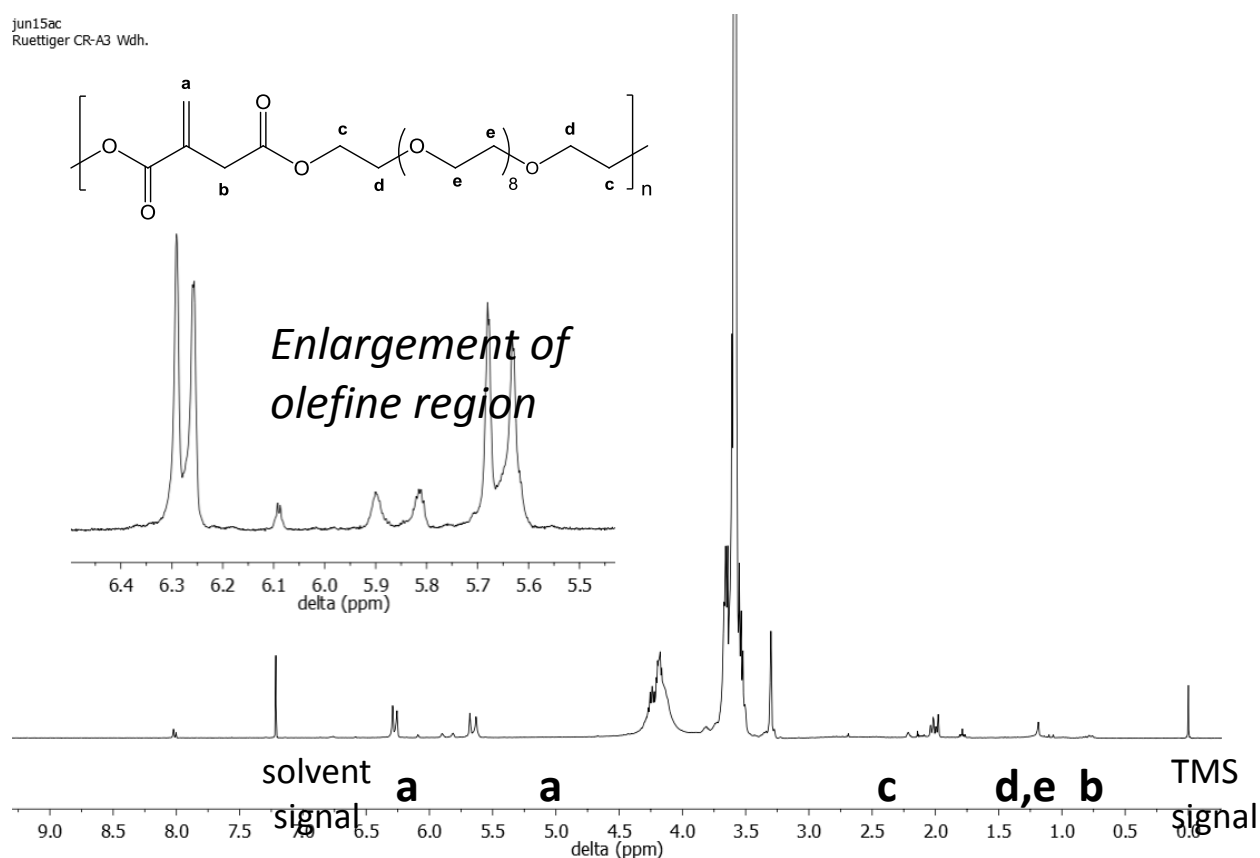


Figure 3: ¹H-NMR (300 MHz) of A3 in CDCl₃.

Solubility Tests

Solvent	Solubility of Product A3
H ₂ O	good
THF	very good
CHCl ₃	very good
EtOH	very good
MeOH	good

The solubility of product A3 increases with hydrophobicity of the solvent. But it is still good soluble in water and methanol. This indicates that alkene cross linking during the synthesis did not occur and a linear polymer was formed.

Batch A4

Date: 11/06/15 – 12/06/15

Lab members: FR, SJ, AW, SZ

Preparation process:

A 20 mL snap-cap vial was loaded with 1.912 g (4.779 mmol, 1 eq.) PEG-400, 0.622 g (4.781 mmol, 1 eq.) itaconic acid and capped with a septum. The snap-cap vial was flushed with argon for 5 minutes and heated up to 145 °C with an oil bath. The snap-cap vial was almost complete dipped into the oil bath to avoid crystallization on the cold glass surface. The formed colorless melt was then stirred under slight vacuum for 24 h. Afterwards the orange-colored viscous liquid was cooled down to room temperature.

Visual Observations:

The orange-colored product was more viscous than A1 and A2. Viscosity and color were comparable with A3. For a picture of A4 see appendix.

Analysis:

The product was analyzed by differential scanning calorimetry (DSC) to identify the glass transition (T_g) temperature. The T_g of A3 is -49.1 °C.

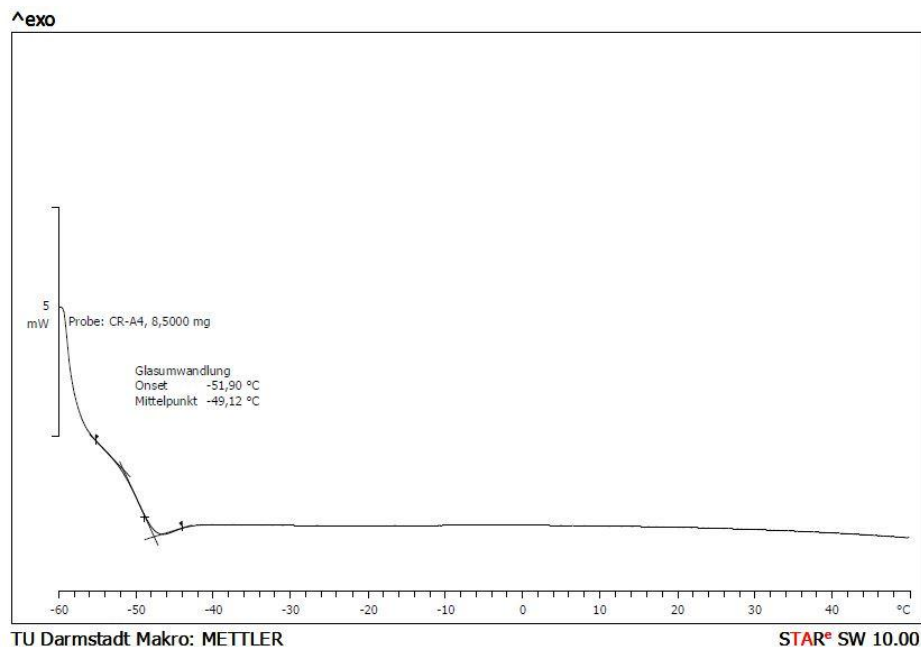


Figure 4: DSC- Plot of A4. The T_g of A4 is -49.1 °C

^1H -NMR

The product was analyzed by ^1H -NMR to ensure that double bonds are retained in the product.

Figure 5 shows the spectrum with an enlargement of the olefin region.

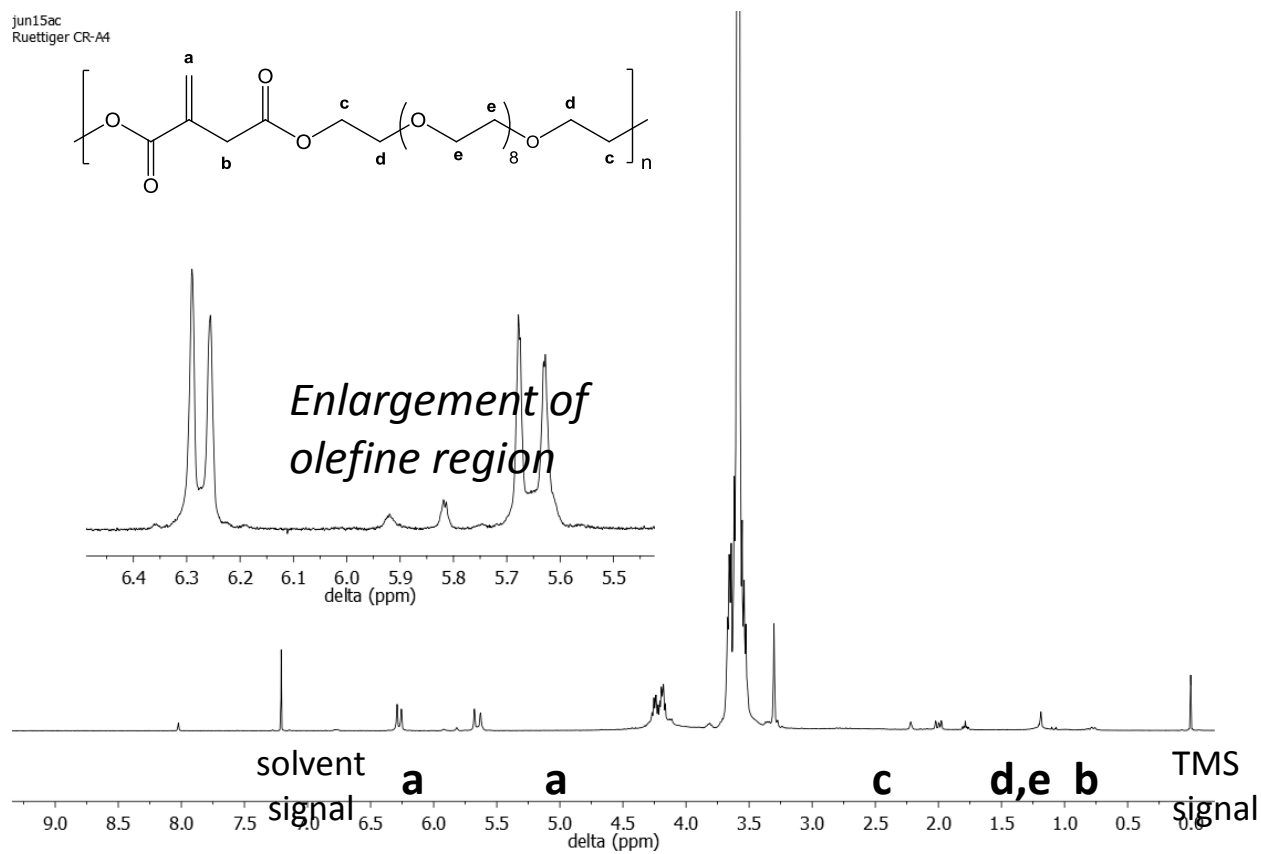


Figure 5: ^1H -NMR (300 MHz) of A3 in CDCl_3 .

Batch A10

Date: 16/07/15 – 17/07/15

Lab members: FR, AW

Preparation process:

A 20 mL snap-cap vial was loaded with 2.2750g (5.688 mmol, 1 eq.) PEG-400, 0.7388 g (5.679 mmol, 1 eq.) itaconic acid and capped with a pierced aluminium foil. The mixture was heated up to 145 °C with an oil bath under atmospheric conditions. No vacuum was applied and the mixture was stirred under atmospheric conditions at 145 °C for 21 h. The brown-colored viscous liquid was cooled down to room temperature.

Visual Observations:

The brown-colored product was more viscous than A8 but less viscous than A7 and A9.

Analysis:

The product was analyzed by differential scanning calorimetry (DSC) to identify the glass transition (T_g) temperature. The T_g of A10 is -46.9 °C.

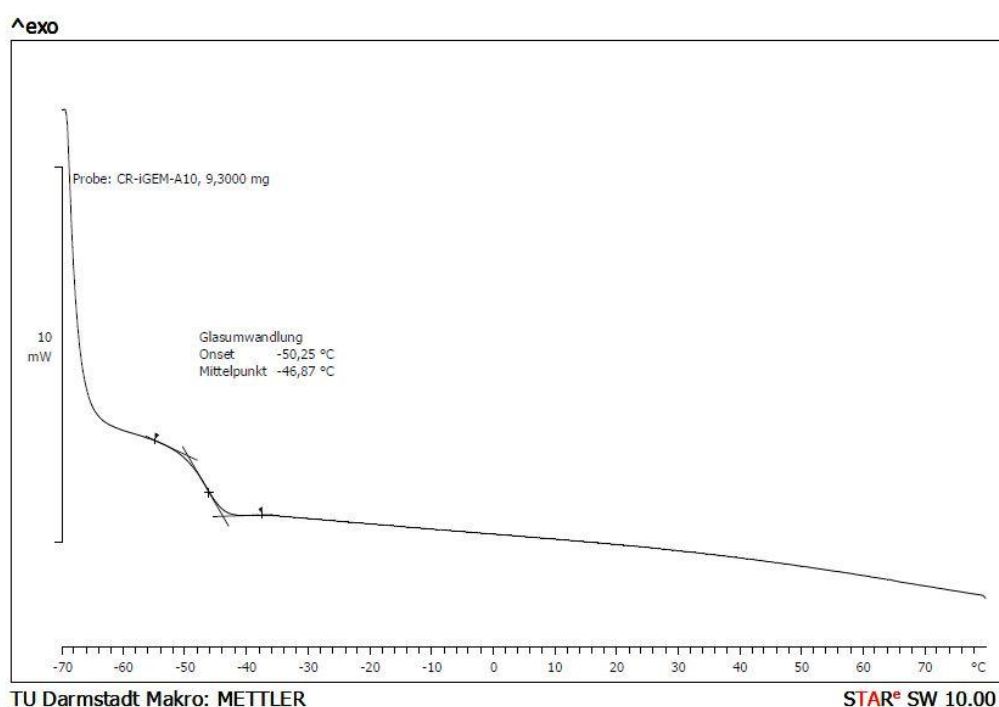
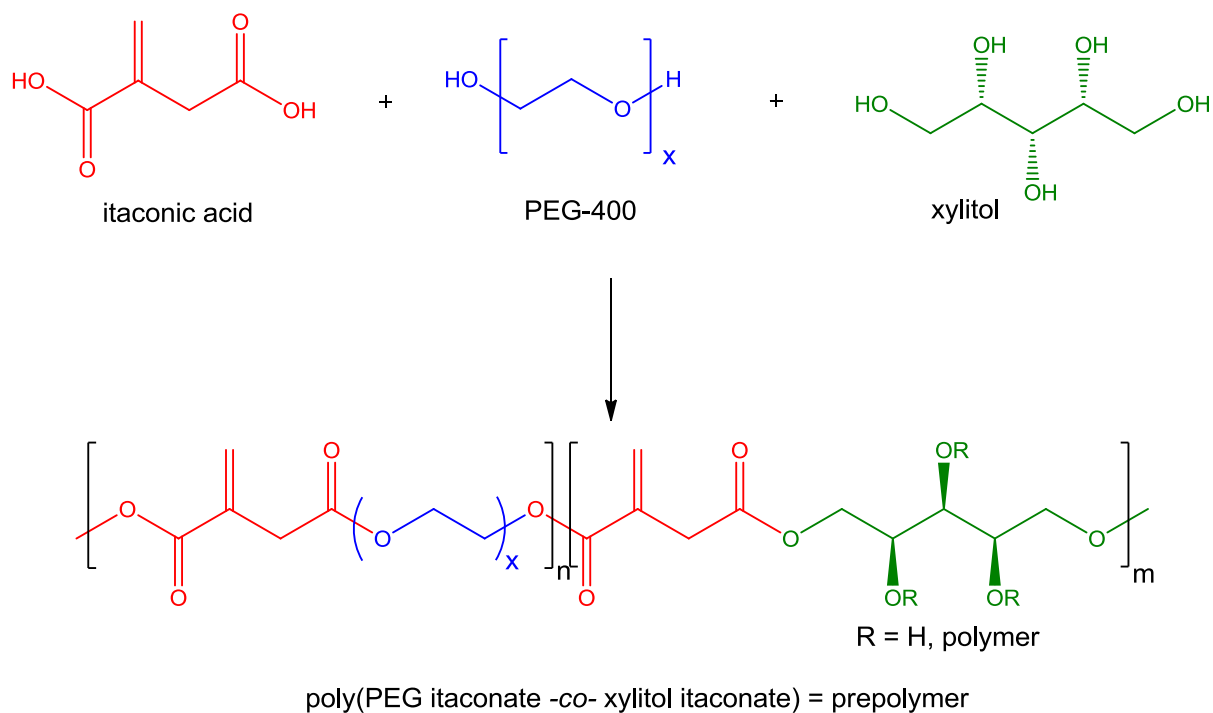


Figure 6: DSC- Plot of A10. The T_g of A10 is -46.9 °C.

Thermal polyesterification of itaconic acid , polyethylene glycol 400 and xylitol



Polymers were prepared in dependence on the experimental procedures of *Barrett et al.*^[1]

The procedure requires exact masses. Therefore an analytical balance (4 decimal places) was used and if not otherwise stated PEG as well as itaconic acid and xylitol were pre-dried before use on a high vacuum line ($< 10^{-3}$ mbar) overnight.

Batch A5

Date: 30/06/15 – 01/07/15

Lab members: FR, SJ, AW, SZ

Preparation process:

A 20 mL snap-cap vial was loaded with 0.997 g (2.491 mmol, 0.43 eq.) PEG-400, 0.3833 g (2.519 mmol, 0.44 eq.) xylitol, 0.7503 g (5.767 mmol, 1 eq.) itaconic acid and capped with a pierced aluminium foil. The mixture was heated up to 145 °C with an oil bath under atmospheric conditions. No vacuum was applied and the mixture was stirred under atmospheric conditions at 145 °C for 17.5 h. The orange-colored gel was cooled down to room temperature.

Visual Observations:

The orange-colored product was a ductile gel. For a picture of A5 see appendix.

Analysis:

Solubility Tests

Solvent	Solubility of Product A5
H ₂ O	no
THF	no
Aceton	no
EtOH	no
MeOH	no

The gel was not soluble in any solvent. Even by applying ultrasound no dissolving occurred. This is probably due to high cross linking with xylitol.

Batch A6

Date: 30/06/15 – 01/07/15

Lab members: FR, SJ, AW, SZ

Preparation process:

A 20 mL snap-cap vial was loaded with 1.2775 g (3.194 mmol, 0.58 eq.) PEG-400, 0.2038 g (1.339 mmol, 0.25 eq.) xylitol, 0.7099 g (5.457 mmol, 1 eq.) itaconic acid and capped with a pierced aluminium foil. The mixture was heated up to 145 °C with an oil bath under atmospheric conditions. No vacuum was applied and the mixture was stirred under atmospheric conditions at 145 °C for 17.5 h. The orange-colored viscous liquid was cooled down to room temperature.

Visual Observations:

The orange-colored product was a ductile gel. For a picture of A6 see appendix.

Analysis:

Solubility Tests

Solvent	Solubility of Product A6
H ₂ O	no
THF	no
Aceton	no
EtOH	no
MeOH	no

The gel was not soluble in any solvent. Even by applying ultrasound no dissolving occurred. This is probably due to high cross linking with xylitol.

Batch A8

Date: 16/07/15 – 17/07/15

Lab members: FR, AW

Preparation process:

A 20 mL snap-cap vial was loaded with 3.4567 g (8.642 mmol, 0.90 eq.) PEG-400, 0.0973 g (0.640 mmol, 0.067 eq.) xylitol, 1.2492 g (9.602 mmol, 1 eq.) itaconic acid and capped with a pierced aluminium foil. The mixture was heated up to 145 °C with an oil bath under atmospheric conditions. No vacuum was applied and the mixture was stirred under atmospheric conditions at 145 °C for 21 h. The orange-colored viscous liquid was cooled down to room temperature.

Visual Observations:

The orange-colored product was a viscous liquid. A8 was less viscous than A10, A7 and A9.

Analysis:

The product was analyzed by differential scanning calorimetry (DSC) to identify the glass transition (T_g) temperature.

The T_g of A8 is -44.8 °C.

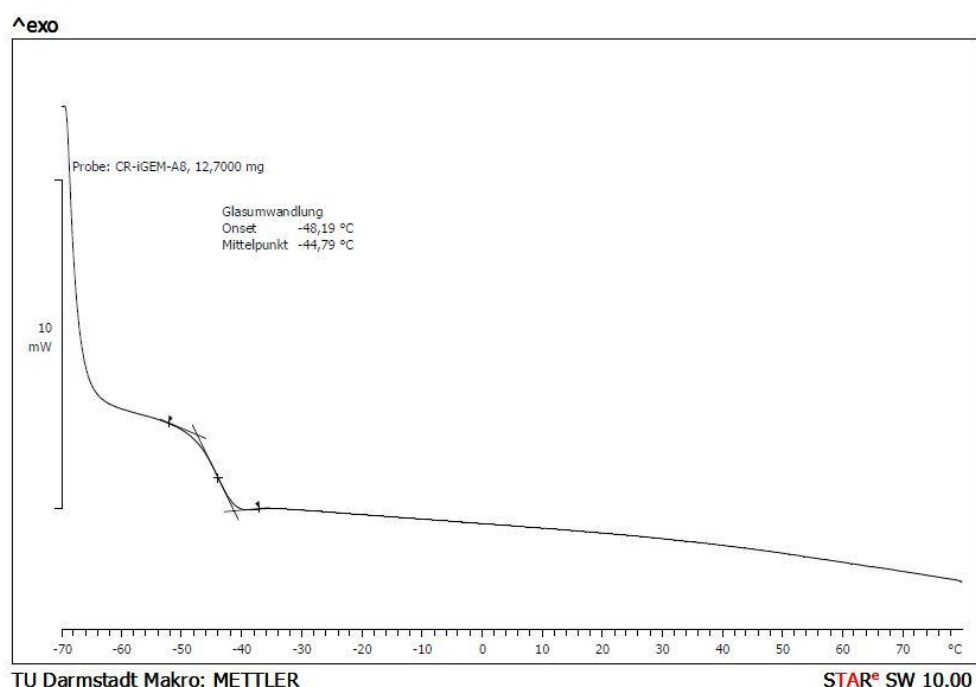


Figure 7: DSC- Plot of A8. The T_g of A8 is -44.8 °C.

Batch A9

Date: 16/07/15 – 17/07/15

Lab members: FR, AW

Preparation process:

A 20 mL snap-cap vial was loaded with 1.5400 g (3.850 mmol, 0.95 eq.) PEG-400, 0.0205 g (0.135 mmol, 0.033 eq.) xylitol, 0.5257 g (4.041 mmol, 1 eq.) itaconic acid and capped with a pierced aluminium foil. The mixture was heated up to 145 °C with an oil bath under atmospheric conditions. No vacuum was applied and the mixture was stirred under atmospheric conditions at 145 °C for 21 h. The brown-colored viscous liquid was cooled down to room temperature.

Visual Observations:

The brown-colored product was a viscous liquid. A9 was more viscous than A7, A10 and A8.

Analysis:

The product was analyzed by differential scanning calorimetry DSC to identify the glass transition (T_g) temperature. The T_g of A9 is -44.4 °C.

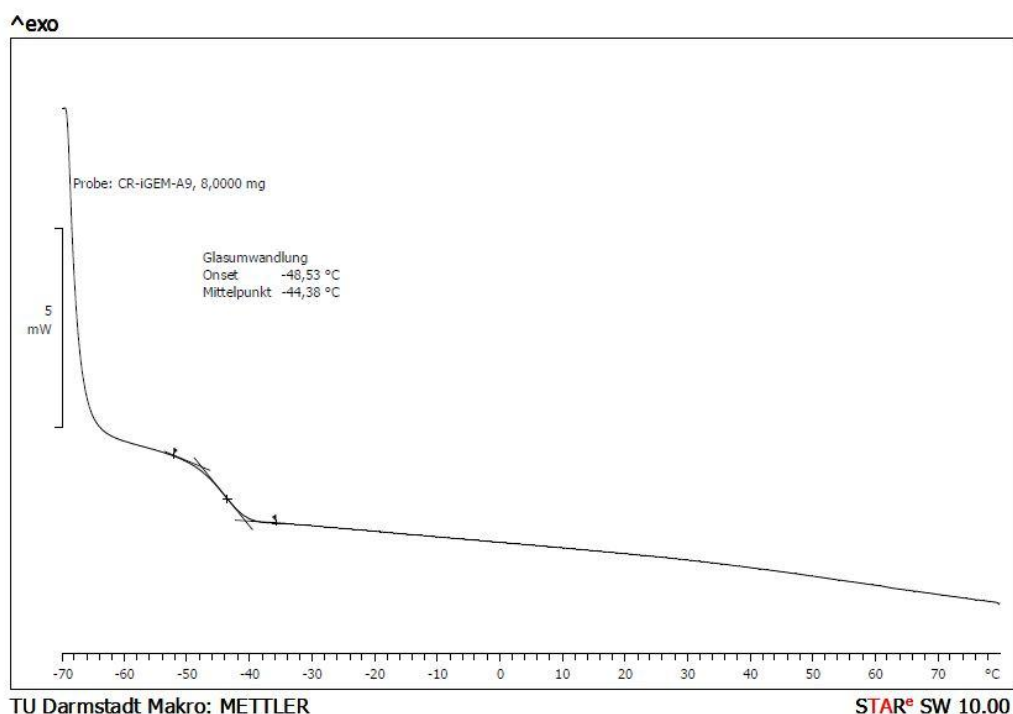
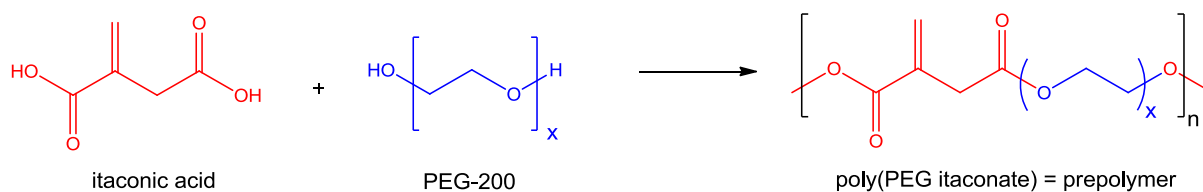


Figure 8: DSC- Plot of A9. The T_g of A9 is -44.4 °C.

Thermal polyesterification of itaconic acid (IA) and polyethylene glycol 200 (PEG-200)



Polymers were prepared in dependence on the experimental procedures of *Barrett et al.*^[1]

The procedure requires exact masses. Therefore an analytical balance (4 decimal places) was used and if not otherwise stated PEG as well as itaconic acid were pre-dried before use on a high vacuum line ($< 10^{-3}$ mbar) overnight.

Batch A7

Date: 16/07/15 – 17/07/15

Lab members: FR, AW

Preparation process:

A 20 mL snap-cap vial was loaded with 3.0745 g (15.373 mmol, 1 eq.) PEG-200, 2.000 g (15.373 mmol, 1 eq.) itaconic acid and capped with a pierced aluminium foil. The mixture was heated up to 145 °C with an oil bath under atmospheric conditions. No vacuum was applied and the mixture was stirred under atmospheric conditions at 145 °C for 21 h. The orange-colored viscous liquid was cooled down to room temperature.

Visual Observations:

The orange-colored product was a viscous liquid.

Analysis:

The product was analyzed by differential scanning calorimetry (DSC) to identify the glass transition (T_g) temperature. The T_g of A7 is -37.1 °C.

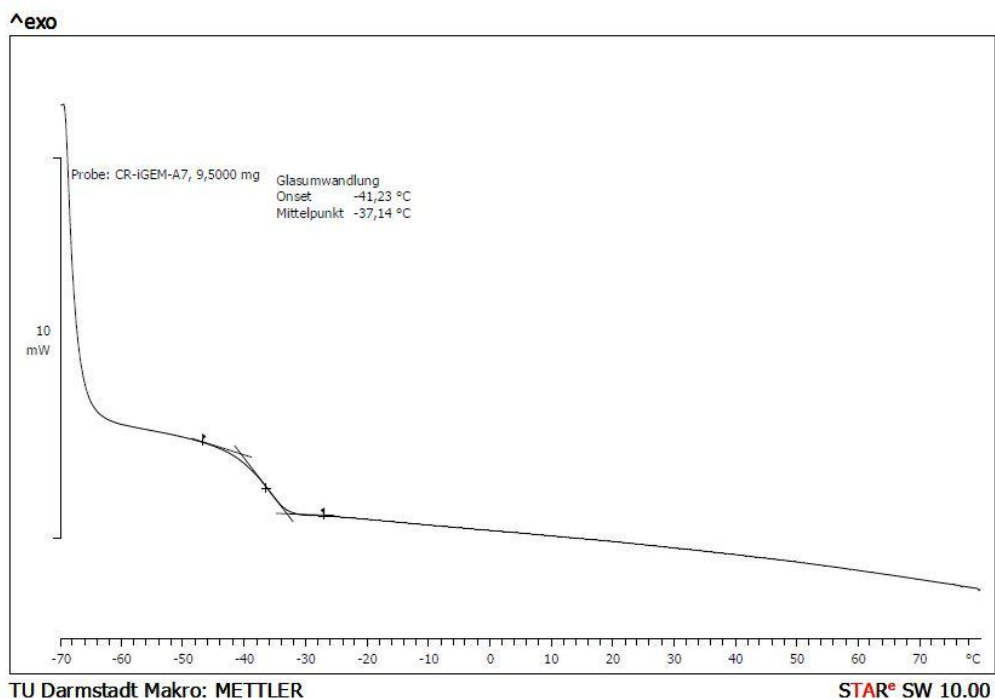


Figure 9: DSC- Plot of A7. The T_g of A7 is -37.1 °C.

Batch A11

Date: 28/07/15 – 30/07/15

Lab members: FR, SJ

Preparation process:

A 20 mL snap-cap vial was loaded with 12.3079 g (61.540 mmol, 1 eq.) PEG-200, 8.0030 g (61.514 mmol, 1 eq.) itaconic acid and capped with a pierced aluminium foil. The mixture was heated up to 145 °C with an oil bath under atmospheric conditions. No vacuum was applied and the mixture was stirred under atmospheric conditions at 145 °C for 42 h. The orange-colored viscous liquid was cooled down to room temperature.

Visual Observations:

On the surface of the orange-colored viscous liquid a rigid gel has formed. For a picture of A11 see appendix.

Analysis:

The product was analyzed by differential scanning calorimetry DSC to identify the glass transition (T_g) temperature. The T_g of A11 is -37.4 °C.

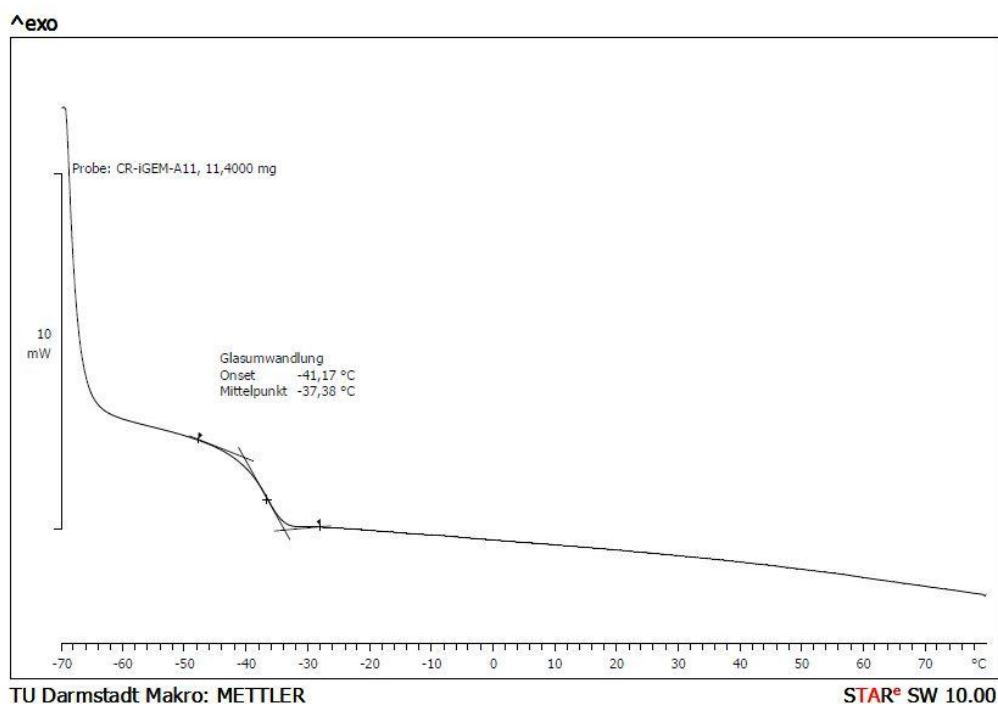


Figure 10: DSC- Plot of A11. The T_g of A11 is -37.4 °C.

Batch A12

Date: 28/07/15 – 30/07/15

Lab members: FR, SJ

Preparation process:

A 20 mL snap-cap vial was loaded with 12.2955 g (61.478 mmol, 1 eq.) PEG-200 and 8.0025 g (61.511 mmol, 1 eq.) itaconic acid. For this experiment itaconic acid was not pre-dried under high vacuum. The vial was capped with a pierced aluminium foil and the mixture was heated up to 145 °C with an oil bath under atmospheric conditions. No vacuum was applied and the mixture was stirred under atmospheric conditions at 145 °C for 42 h. The orange-colored viscous liquid was cooled down to room temperature.

Visual Observations:

On the surface of the orange-colored viscous liquid a rigid gel has formed. For a picture of A12 see appendix.

Analysis:

The product was analyzed by differential scanning calorimetry (DSC) to identify the glass transition (T_g) temperature. The T_g of A12 is -37.1 °C.

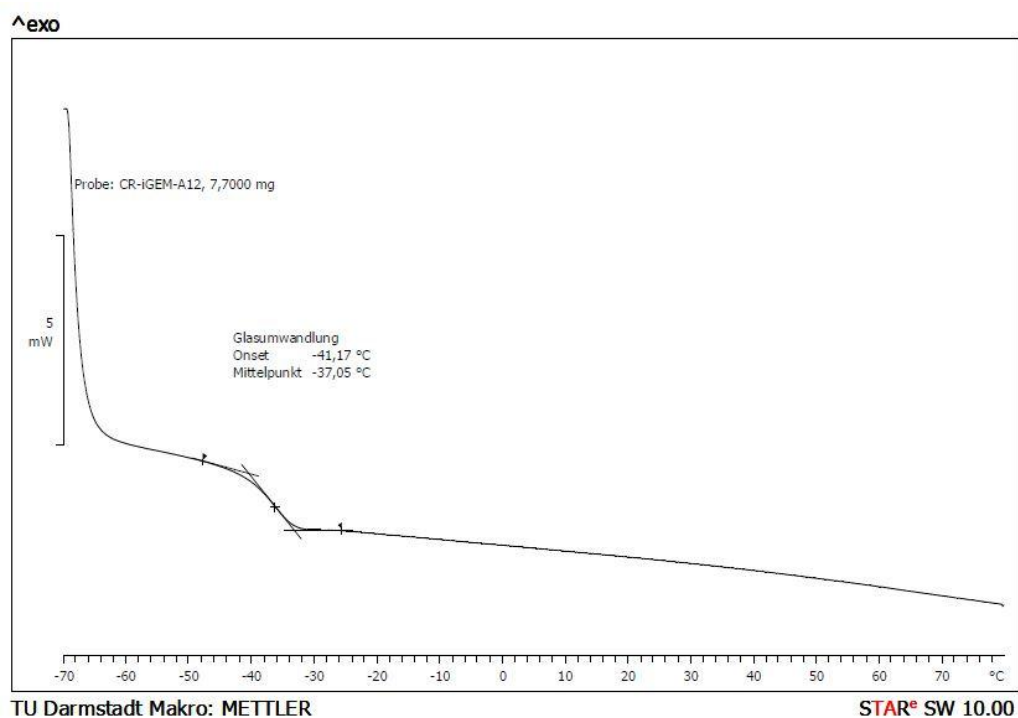


Figure 11: DSC- Plot of A12. The T_g of A12 is -37.1 °C.

Batch A13

Date: 12/08/15 – 13/08/15

Lab members: SJ

Preparation process:

A 500 mL round bottom flask was loaded with 90.88 g (454.4 mmol, 1 eq.) PEG-200 and 59.12 g (454.4 mmol, 1 eq.) itaconic acid. For this experiment itaconic acid was not pre-dried under high vacuum. The flask was capped with a pierced aluminium foil and the mixture was heated up to 145 °C with an oil bath under atmospheric conditions. No vacuum was applied and the mixture was stirred under atmospheric conditions at 145 °C over night. The orange-colored viscous liquid was cooled down to room temperature.

Visual Observations:

At 143 °C IA was completely dissolved and a slightly yellow melt has formed. The produced water condensed partially on the wall of the flask. For a picture of A13 during and after synthesis see appendix.

Analysis:

The product was analyzed by differential scanning calorimetry (DSC) to identify the glass transition (T_g) temperature. The T_g of A13 is -40.8 °C.

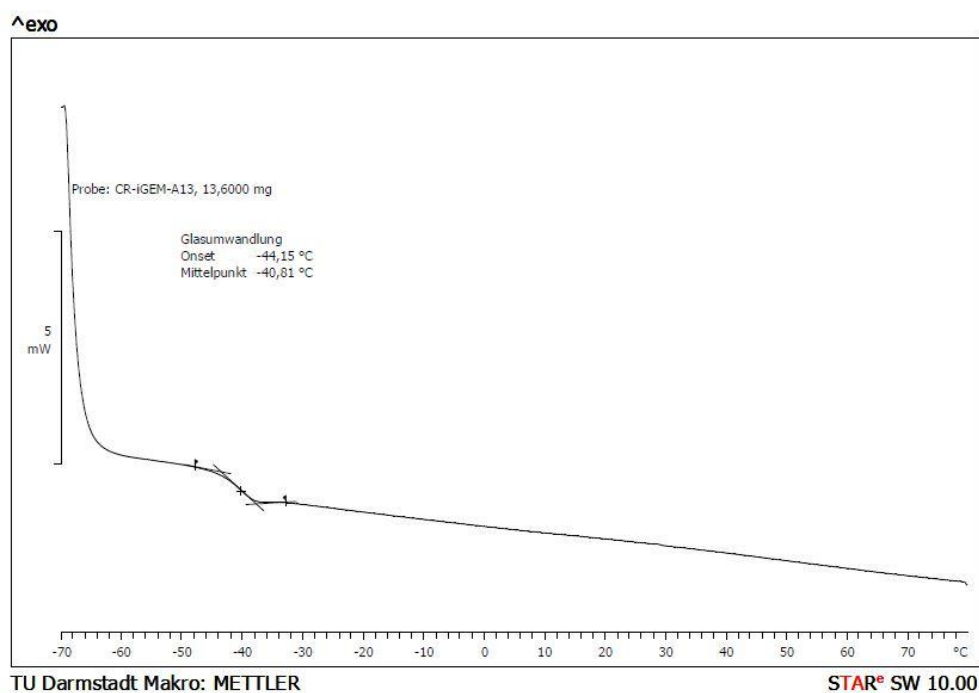


Figure 12: DSC- Plot of A13. The T_g of A13 is -40.8 °C.

Batch A15

Date: 18/08/15 – 19/08/15

Lab members: SJ, AW, SZ

Preparation process:

A 1 L round bottom flask was loaded with 302.9 g (1.51 mol, 1 eq.) PEG-200 and 197.0 g (1.51 mol, 1 eq.) itaconic acid. For this experiment itaconic acid was not pre-dried under high vacuum. The flask was capped with a pierced aluminium foil and the mixture was heated up to 145 °C with an oil bath under atmospheric conditions. No vacuum was applied and the mixture was stirred under atmospheric conditions at 145 °C for 31 h. The orange-colored viscous liquid was cooled down to room temperature.

Visual Observations:

After 24 h a viscous orange-colored liquid has formed. The loss of mass during the synthesis does not correspond to the calculated mass loss due to produced and evaporated water (2 eq. water by high conversion). A sample for DSC analysis was taken and the mixture was stirred for 7 h. Afterwards another sample for DSC analysis was taken. For a picture of A15 see appendix.

Analysis:

The product was analyzed by differential scanning calorimetry (DSC) to identify the glass transition (T_g) temperature. The T_g of A15 after 24 h at 145 °C is -39.9 °C. The T_g of A15 after 31 h at 145 °C is -37.9 °C.

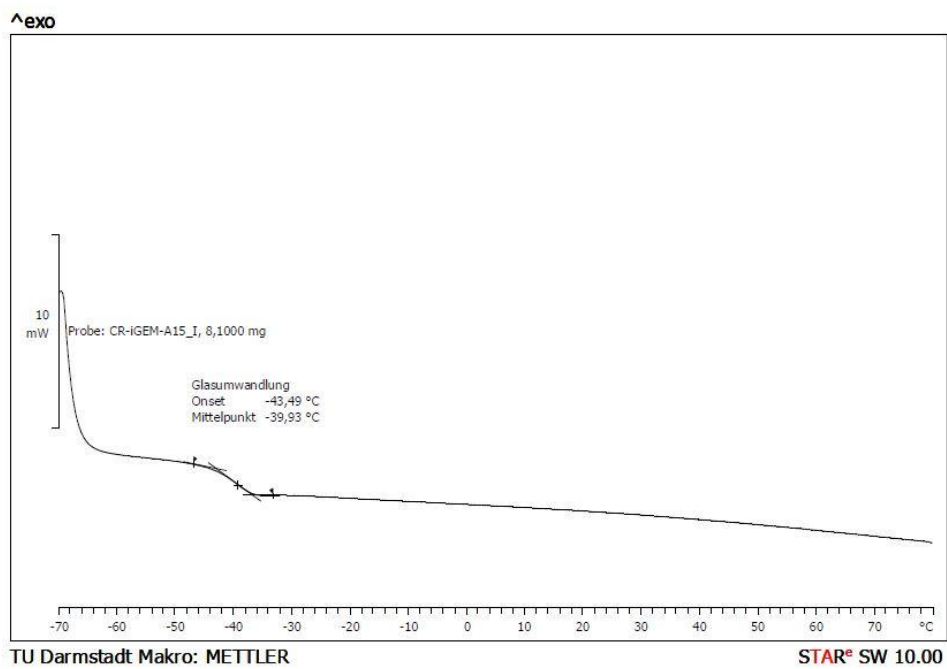


Figure 13: DSC- Plot of A15 after 24 h at 145 °C. The T_g of A15 is -39.9 °C.

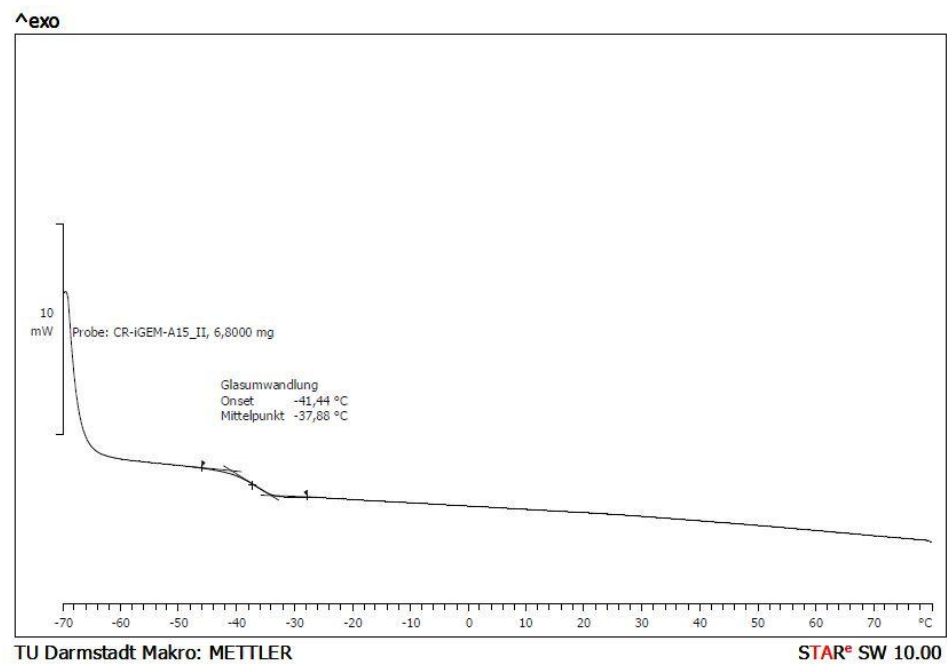


Figure 14: DSC- Plot of A15 after 31 h at 145 °C. The T_g of A15 is -37.9 °C.

Analysis of the prepolymers with GPC

The prepolymers A10, A12, A13 and A15 were analyzed by Gel permeation chromatography (GPC) to detect the average molar mass.

Method:

The metering was performed with the PSS Suprema HS column connect to a RID detector. NaNO_3 (0.1 mol/l) was used as eluent by a flow rate of 1 ml/min and polyacrylic acid as polymer standard for the calibration.

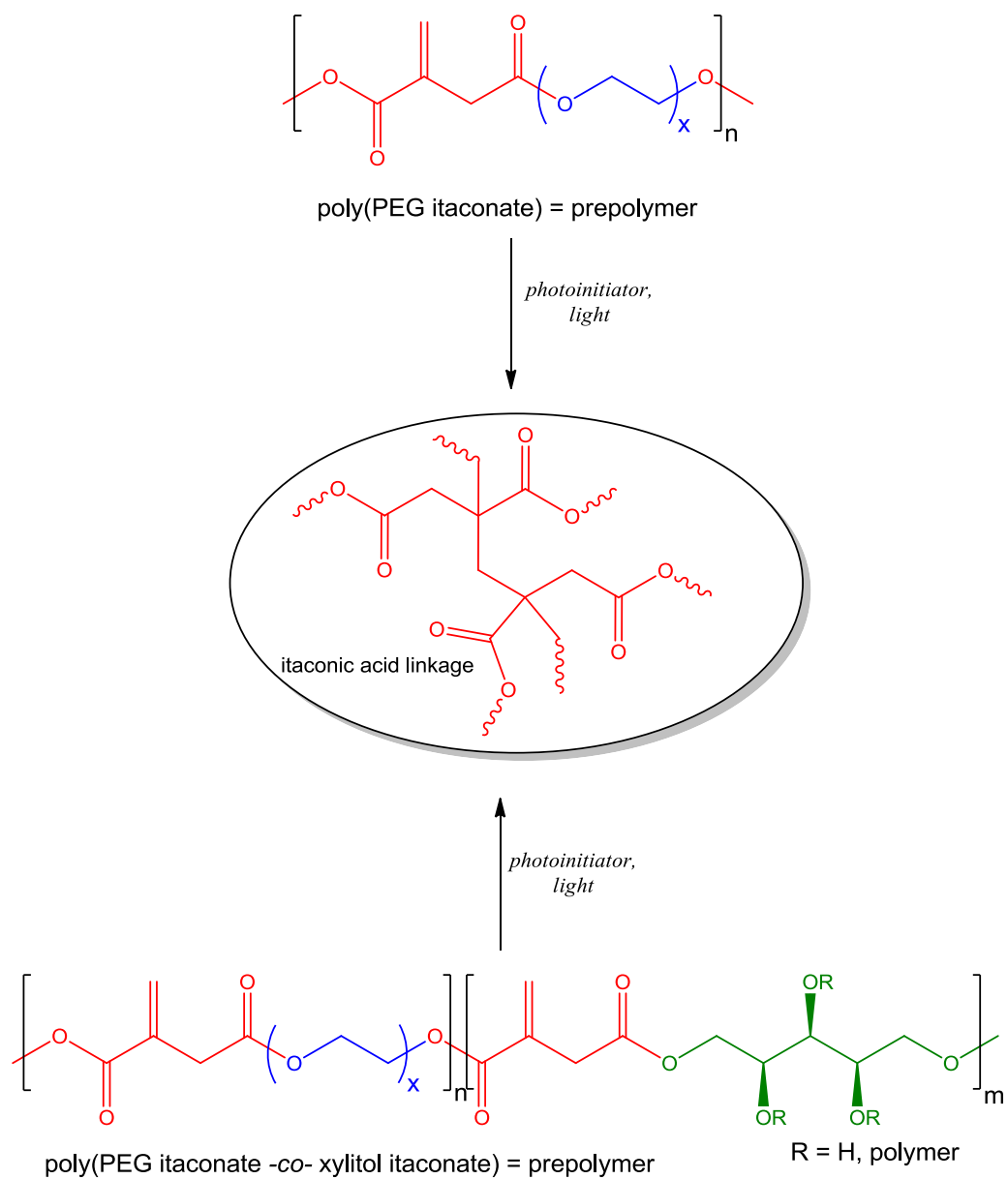
Results:

All assays were smaller than the smallest polymer standard of 1250 g/mol. Therefore the average molar mass was not detectable.

Overlaying the assays allows the comparison of the different prepolymers. Thereby, one can see that the polymers A 12, A13 and A15 are very similar and only A10 has a different curve. This reflects the composition of the prepolymers. While A10 is composed of PEG 400 and IA, the prepolymers A12-A15 are composed of PEG 200 and IA.

Figure 15: GPC-Plot of A10 (light blue), A12 (blue), A13 (yellow) and A15 (black).

Curing of prepolymers



Prepolymers were mixed with different amounts of Irgacure 1173 photoinitiator and streaked on microscope glass slides. The slides were placed for different periods of time under UV-light 254 nm.

Prepoly- mer/ sample	Photoinitiator [wt-%]	Curing time (254 nm)		
		5 min	10 min	15 min
PEG-400	1			
A2	1			
A3	2.3	-	-	currugation, soft
A4	1.8	-	-	currugation, soft
A7	2	-	-	solid
A8	2	-	-	gel
A9	2	-	-	gel
A10	2	-	-	gel
A11	0	currugation, soft	currugation, soft	currugation, soft
A11	0.5	currugated skin	currugated skin	currugated skin
A11	1	currugated skin	currugated skin	currugated skin
A11	2	currugated skin	currugated skin	currugated skin
A11	4	almost solid, plain	plain solid	plain solid
A11	9	plain solid	plain solid	plain solid
A12	0	currugation, soft	currugation, soft	currugation, soft
A12	0.5	plain skin	plain skin	plain skin
A12	1	plain skin	plain skin	plain skin
A12	2	plain skin	plain skin	plain skin
A12	4	almost solid, plain	plain solid	plain solid
A12	9	plain solid	plain solid	plain solid

By UV-curing of A8 on a polystyrene petri dish an interesting effect was observed. The hydrophilic hydroxyl groups of xylitol lead to formation of prepolymer drops due to the hydrophobic polystyrene surface of the petri dish. This effect is interesting because it could prevent adhesion of the polymer on the basin of the 3D printer during UV curing. Unfortunately A8 cannot be cured by UV light, even not with 6 % DAROCUR 1173. For a picture see appendix.

References:

- [1] Barrett et al., *Macromolecules* **2010**, *43*, 9660-9667.

Appendix

Pictures of prepolymers during and after synthesis

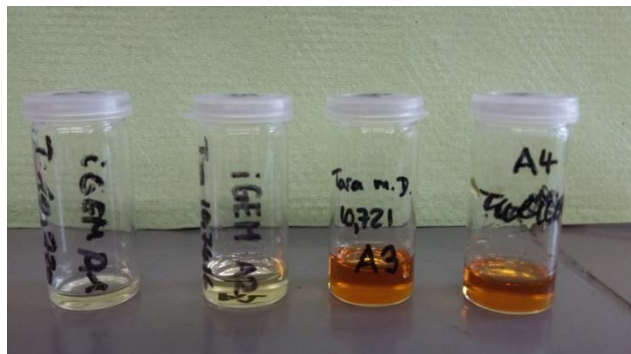


Figure 15: From left to right A1 – A4 after synthesis.

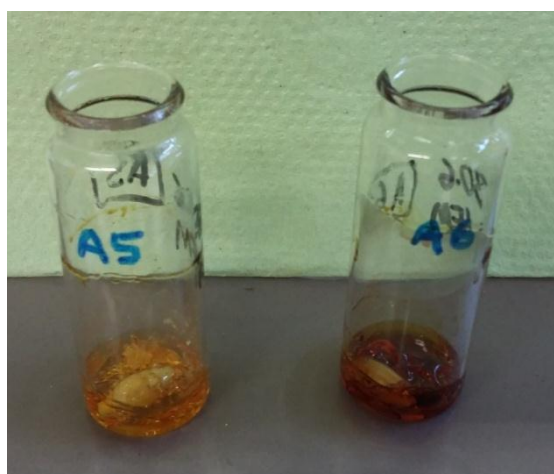


Figure 16: A5 and A6 after synthesis.



Figure 17: From left to right A12 – A11 after synthesis.



Figure 18: A13 and A14 during (left) and after synthesis (right).



Figure 19: A15 during the heating process. At 131 °C no melt has formed and the white itaconic acid can still be seen.

Pictures of cured prepolymers

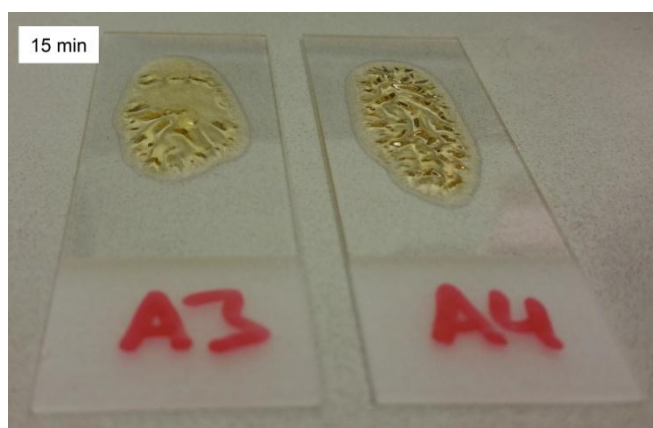


Figure 20: UV-curing of A3 and A4 after 15 min.

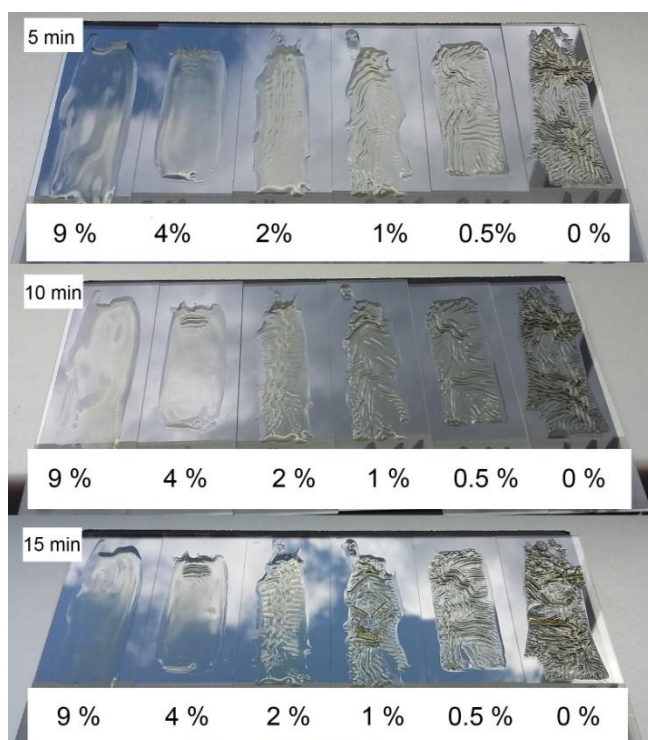


Figure 21: UV-curing of A11 after 5, 10 and 15 min.

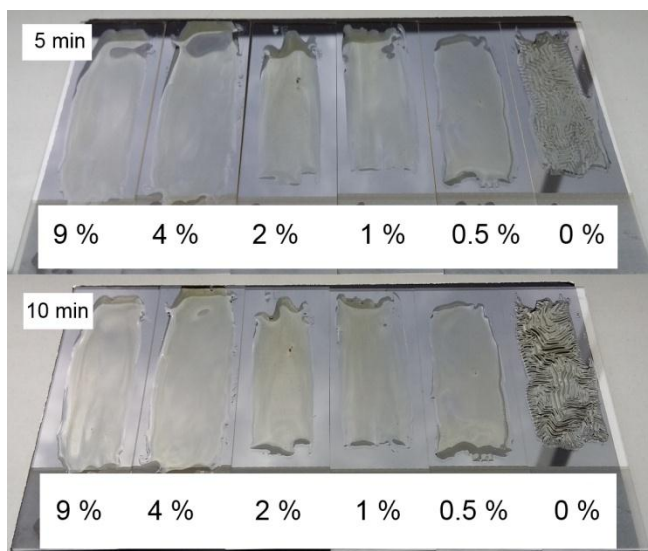


Figure 22: UV-curing of A12 after 5 and 10 min.

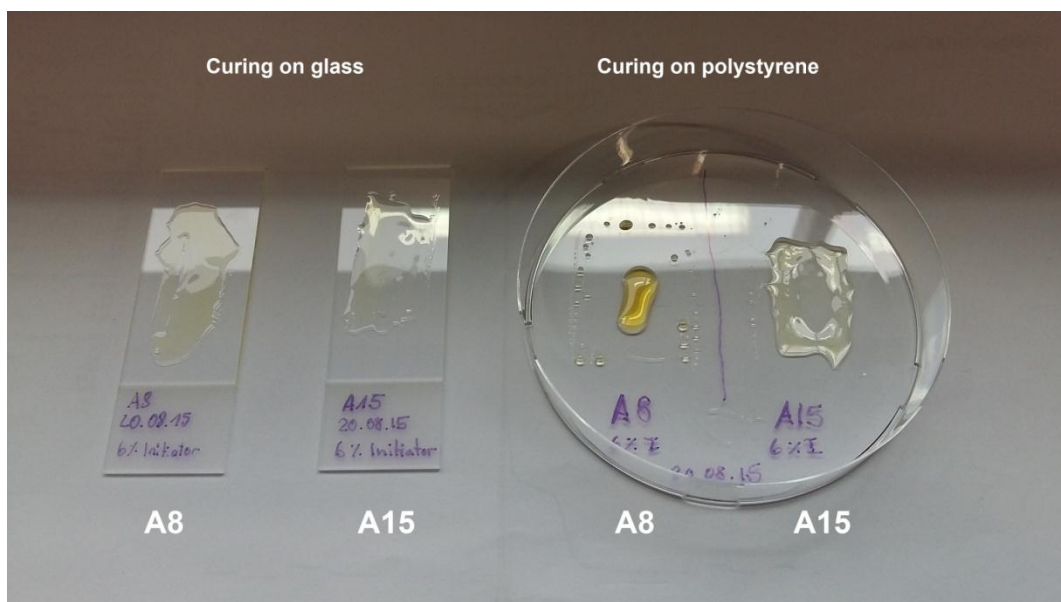


Figure 23: UV-curing of A8 and A15 on glass and on a polystyrene petri dish after 5 min 254 nm. 6 % DAROCURE 1173 was used.